

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No. Not Yet Known
Filed: Not Yet Known
Continuation-in-Part of: Serial No. 08/781,308
Inventors: Ellington M. Beavers et al
Title: METHOD OF MAKING FREE ACIDS
FROM POLYSACCHARIDE SALTS
File No.: 281-28

DECLARATION OF ELLINGTON M. BEAVERS UNDER RULE 132

Ellington M. Beavers hereby declares as follows:

1. I am one of the named joint inventors in the above-identified application, which is a continuation-in-part of Serial No. 08/781,308. I make this Declaration in support of the patentability of the claims of this CIP application.

2. In the prior application, Serial No. 08/781,308, the Examiner allowed the method claims, but rejected the product-by-process claims over several references which purported to disclose free hyaluronic acid. The purpose of this Declaration is to establish, by experimental evidence, that the product produced by the process of the present invention is significantly and patentably different from the products made by processes disclosed in the prior art. The following experiments describe the evaluation of coatings made from a) sodium hyaluronate, b) free hyaluronic acid made according to the present invention, and c) free hyaluronic acid made according to methods of the prior art.

3. In the first experiment, cosmetic grades of sodium hyaluronate were obtained from four commercial suppliers and were designated as samples B, K, P, and T. Each of these was weighed into round bottles with a calculated amount of distilled water to produce viscous solutions of 0.5% con-

centration. All were clear, colorless and free of particulate matter. Following the teachings of U.S. Patent Nos. 4,801,475, 5,023,114, and 5,037,677, cited in the above application, bilaminar coatings were applied to a neutral, toxicologically clean plastic billet. The coatings were cured, and the coated billets were sent to a commercial toxicology laboratory for tests of hemolysis, i.e. the tendency of the coatings to destroy blood cells, using rabbit blood, and for cytotoxicity, using an elution test. The results are shown in the table below:

<u>Sample</u>	<u>% Hemolysis</u>	<u>Hemolytic?</u>	<u>Reactivity</u>	<u>Cytotoxic</u>
B	36.8	Yes	Severe (4)	Yes
K	27.3	Yes	Severe (4)	Yes
P	28.2	Yes	Severe (4)	Yes
T	2.45	No	Moderate (3)	Yes

In the above table, the term "reactivity" refers to cytotoxicity. The test surface was placed in contact with a cell culture, and the cytotoxicity was evaluated on a scale of 0-4, with "4" representing the most severe cytotoxicity.

With regard to hemolysis, any value over 5% is considered hemolytic, and all but Sample T were therefore found to be hemolytic in the above test. All four samples were found to be cytotoxic. Therefore, all four samples must be considered unacceptable for use in forming coatings on medical devices for internal use.

4. In the second experiment, another sample of the cosmetic grade of sodium hyaluronate designated T was dissolved in distilled water at a concentration of 0.6%, acidified with hydrochloric acid and transferred to dialysis tubing having a molecular weight cutoff of 3500. It was dialyzed

against frequent changes of distilled water until the pH of the surrounding water reached 5.4. The dialyzate was tested with 5% aqueous silver nitrate and found to be free of chloride ion. The hyaluronic acid was transferred from the dialysis tubing to a sterile bottle and found to have a pH of 3.0. Its solution was colorless, sparkling clear, and somewhat diluted to 0.5% solids content due to reverse osmosis.

When tested as the top-coat in the bilaminar grafted coating described in U.S. Patent Nos. 4,801,475, 5,023,114, and 5,037,677, the coating showed no reactivity in the test for cytotoxicity and zero percent hemolysis. This result shows the effective removal of the toxic factor in sample T that caused 2.45% hemolysis and moderate reactivity in the cytotoxicity test, in the table shown in Paragraph 3, above. In other words, this product would be suitable for use on a medical device to be implanted in the body.

5. A further experiment was then conducted, to determine whether a similar improvement could be achieved in the conversion of sodium hyaluronate to hyaluronic acid by the method of the prior art.

Another ten-gram quantity of sample T was dissolved in distilled water at a concentration of 0.6%. It was stirred for one hour with five grams of Amberlite IR-120 (Plus), a strongly acidic gel-type resin in the acid form, and then filtered. The resulting solution of hyaluronic acid showed a pH of 2.8 and was clear and colorless. It was used as the top-coat in a bilaminar grafted coating, as in the previous experiment. When tested, the coating showed hemolysis of 2.5% and was cytotoxic. Thus, the hyaluronic acid produced by this procedure would not be acceptable for use as a coating on a medical device implanted in the body.

The procedure used in this experiment is comparable to those described

in U.S. Patent Nos. 4,589,963 (Cipriano), 4,736,024 (Della Valle), and 5,268,079 (Ochoa Gomez). All of these patents rely on the ionic character of sodium hyaluronate to operate successfully. Thus, in Cipriano, the permselective membranes have anionic functionality (sulfonic or carboxyl anionic substituent groups), allowing the passage of positive sodium ions but repelling the negative hyaluronic anion, thus effecting the claimed salt-splitting. The same dependence on ionic charge applies in the case of Ochoa Gomez, where an applied electromotive force causes the passage or rejection of ions and effects salt-splitting. The same is true of Della Valle, where an anion-exchange resin is used to replace sodium ions with hydrogen ions, causing the formation of a solution of free hyaluronic acid.

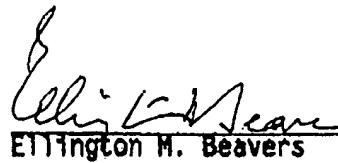
The process conducted in my above-described experiment was analogous to the processes of Cipriano, Della Valle, and Ochoa Gomez, because the strongly acidic gel-type resin (Amberlite IR-120 (Plus)) works in the same manner as a permselective membrane having anionic functionality. That is, the hyaluronic acid is produced by separation of ions, due to the electrical properties of a separation medium, which, in this experiment, was the acidic gel-type resin. In my invention, by contrast, the separation is based not on ionic separation, but on the pore size of a non-ionic, electrically neutral membrane.

6. The above-described experiments show that not all hyaluronic acid is alike. The present invention produces hyaluronic acid which is not hemolytic or cytotoxic, and which is therefore suitable for use in coating medical devices which will be implanted in the body. The patents to Cipriano, Della Valle, and Ochoa Gomez show processes which yield products that are not suitable for internal use in the body.

7. U.S. Patent No. 5,532,221 (Huang) mentions free hyaluronic acid, but says nothing about how the hyaluronic acid is produced. Huang cannot be deemed to suggest the present invention. The mere mention of hyaluronic acid, without a description of the process used to make it, is not a teaching or suggestion of the present process. My experiments have shown that the method used to make the hyaluronic acid greatly affects its usability in the medical field. I am aware of no prior art reference which teaches or suggests my method.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: July 17, 1998


Ellington M. Beavers